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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) Pesticide exposure has been associated with increased Parkinson's disease (PD) risk. Results from the Honolulu-Asia Aging Study (HAAS) supported by this project showed PD risk doubled in individuals who worked on plantations over 20 years. Others have found high organochlorine levels in brains from decedents with PD relative to controls and dopaminergic neuron toxicity has been demonstrated in vitro but not proven in humans. This supplement aim was to measure organochlorine levels in all brains from the HAAS archive and to examine the association of brain organochlorine levels with numerous clinical and pathological endpoints obtained through prior and ongoing research. Frozen occipital blocks from 421 brains were sent to Dr. E.D. Pellizzari of Research Triangle Institute for organochlorine level assays. These are complete. Preliminary analyses demonstrate that a measurable a-chlordane level was significantly more frequently in brains with Lewy bodies (including PD cases) than in those without Lewy bodies (p=0.03). Additional analyses investigating relationships between brain organochlorine levels and substantia nigra neuron densities, striatal dopamine levels, brain glial fibrillary acidic protein levels, and cognitive ability are planned.

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Introduction

One of the most intriguing developments in the search for a cause of Parkinson's disease (PD) during the last decade has been the association of pesticide exposure with increased risk of disease. Epidemiologic studies have linked rural living, farming and other agricultural occupations to a higher risk of developing PD in humans.(1-6) Recent work supported by this DoD funded project from the longitudinal Honolulu-Asia Aging Study indicates that study participants who worked on sugar or pineapple plantations for more than 20 years in Hawaii had approximately double the risk of developing PD compared to those who never worked on a plantation(7) Finally, a role for pesticide exposure in PD has also been supported by autopsy studies reporting significantly higher levels of organochlorine compounds in brains from PD cases compared to AD cases or normal controls(8;9;9) It is hypothesized that these compounds may have a toxic effect on dopaminergic neurons. This has been demonstrated in vitro(10;11) but has not been proven in humans. In Hawaii, the organochlorine DDT (metabolized in the body to DDE and DDD) was in very common use in the agricultural, industrial, and home settings in the state against a variety of pests including mosquitoes. Chlordane (termite control) and mirex (ant control) were commonly used around homes. Methoxy, dieldrin, aldrin, and heptachlor had more specific agricultural / industrial uses. Consequently, a pilot study was performed as part of the original project that measured organochlorine compounds in frozen brain tissue in 15 HAAS brains from participants with the highest exposure history to pesticides. Organochlorine pesticide and lipid analysis were performed on each of these at the Analytical and Chemical Sciences lab, Research Triangle Institute, Research Triangle Park, NC under the direction of Dr. E. D. Pellizzari.

Results of this pilot indicated that several samples had levels of one or more organochlorine compounds higher than 10 ppb, levels that would be considered significantly high if found in the blood. DDE, g-chlordane, and methoxy were especially prominent. It is safe to say that detectable levels of these substances in any individual brain reflect exposure to those compounds during the time that the compounds were in use. These chemicals remain detectable in tissues for many years. In some cases there are more than 30 years between the exposure (ie to plantation work or self report of exposure to pesticides) and death. Therefore, these data can provide a quantifiable exposure measurement that, along with occupational data, will allow calculation of the age at the time of exposure. Accordingly, our aims in this supplement were to determine levels of organochlorine compounds in the brains of all available autopsy cases in our brain bank. This supplement seeks not just to replicate the findings of others regarding high organochlorine levels in the brains of deceased PD patients, but to also utilize the enormous HAAS database to examine the association of brain levels of organochlorine compounds with numerous clinical and pathological endpoints that have been obtained through work already accomplished.

Body

Research accomplishments are listed below associated with each task of the statement of work.

1. **Perform organochlorine measurements on all 485 brains in the HAAS brain bank plus the additional 15 that will accrue in the months prior to sending the samples to Research Triangle Park.** There were 421 samples of frozen occipital lobe shipped to Dr. E.D. Pellizzari of Research Triangle Institute for organochlorine levels in four shipments sent 5/03, 8/03, and 11/03 and 4/04. Our original proposal called for 500 samples, however, frozen occipital tissue was only available for 421. These assays have been completed and the data has been returned to us in the form of four spreadsheets corresponding to the four shipments. The first spreadsheet containing

results for 98 samples has completely undergone data clean-up, while data in the other three spreadsheets are being reviewed by Dr. Ross and Dr. He (biostatistician).

2. **Take advantage of existing data from the HAAS to examine the association of organochlorine brain levels with Parkinson's disease; extrapyramidal motor signs determined by the Unified Parkinson's disease rating scale; Lewy bodies in the brainstem and cortex; neuron counts in the substantia nigra; and striatal dopamine and dopamine metabolite levels. It should be mentioned that all of these variables are currently part of the HAAS database or are being performed as part of projects already funded.** These analyses are complicated and our biostatistician is currently evaluating several possible strategies for demonstrating theses relationships. Preliminary results generated from the first 98 samples compared the prevalence of a measurable level of a-chlordane in the occipital lobe between brains with Lewy bodies (including PD cases) and those without Lewy bodies. Results indicated that significantly more brains with Lewy bodies in the substantia nigra or locus ceruleus have measurable a-chlordane than those without Lewy bodies.

PD/LB	Detectable level or a-chlordane:		
	Yes	No	Total
Yes	15 (58%)	24 (33%)	39
No	11 (42%)	48 (67%)	59
Overall	36 (100%)	72 (100%)	

The p-value for the Chi-square test is 0.03.

3. **Examine the association of brain organochlorine levels with presence of Alzheimer's disease, cognitive function defined by the CASI, brain weight, neuritic plaque counts, and neurofibrillary tangle counts. These variables are also part of the current HAAS database.**
4. **Examine the association of brain organochlorine levels with GFAP levels in frontal, parietal, temporal, and occipital lobes; hippocampus; and striatum (caudate and putamen). GFAP levels are either already available or are being performed for this DoD project and other funded projects.**
5. **Examine the interaction of brain organochlorine levels and presence of the CYP2D6 HhaI polymorphism as a risk factor for PD using available data on the genetic polymorphism.**

Analyses in the early stages for items 2 – 5 pending the final organochlorine level results for the remaining 333 samples. This is in accordance with our timeline as submitted with the original request for the supplement.

Key research Accomplishments

- Organochlorine measurements have been performed on 421 frozen occipital lobe tissue samples by Research Triangle Institute and the data returned in spreadsheet format.
- The prevalence of a measurable amount of a-chlordane is higher in the brains that have Lewy bodies in the substantia nigra or locus ceruleus than in those brains without these lesions (p=0.03).

Reportable Outcomes

- Dr. Diane B. Miller from the National Institute for Occupational Safety and Health presented an abstract entitled "Brain Tissue Analysis in the Honolulu-Asia Aging Study; Pesticides and other Persistent Chemicals" at the 21st International Neurotoxicology Conference held in Honolulu, HI February 10 -14, 2004. Her presentation showed that there are measurable levels of organochlorine compounds in a high proportion of the HAAS brains studied. Abstract is attached (Appendix 1).
- Dr. Edo Pellizzari from Research Triangle Institute presented an abstract entitled, "Method for the Determination of Organochlorine Pesticides in Human Brain Tissue," at the 14th annual conference of the International society of Exposure Analysis held October 17 through 21, 2004. The abstract is attached (Appendix 2).

Conclusions

The majority of analyses for this project are still in the early stages. This work will continue with funding from other projects that will utilize the organochlorine level data. Our first set of analysis will focus on the association of high organochlorine levels with the clinical diagnosis of Parkinson's disease. Next we will use existing neuropathological data to examine the relationship of organochlorine levels to the presence of Lewy bodies in the substantia nigra or locus ceruleus in all 421 brains available from deceased HAAS participants.

We are now performing dopaminergic neuron counts on single sections through the substantia nigra and dopamine / dopamine metabolite levels on frozen samples of the caudate nucleus and putamen from all brains in the HAAS archive as part of a National Institute of Neurological Disorders and Stroke funded project. We plan to directly examine the relationship between organochlorine levels and these two neuropathological markers of PD within brains. The demonstration of high brain levels of organochlorines with low numbers of substantia nigra dopamine neurons and/or low levels of striatal dopamine would provide compelling evidence for a direct toxic effect of organochlorines on the nigrostriatal system in humans.

Another component of the existing DoD supported project is a study of glial fibrillary acidic protein (GFAP) brain levels in Alzheimer's disease and Parkinson's disease. GFAP is produced by astrocytes and is the major constituent of glial filaments that accumulate in response to central nervous system injury in a process called astrogliosis or reactive gliosis.(12;13) Reactive gliosis can be induced by a number of insults to the brain including physical damage, disease, or chemicals. Glial proliferation occurs in Alzheimer's disease and though the mechanism is unknown, it is postulated to be related to inflammatory processes. Inflammation has also been hypothesized to play a role in the pathophysiology of Parkinson's disease and this is currently an important area of research.

Investigators from Honolulu and the National Institute for Occupational Safety and Health (NIOSH) have collaborated to quantify GFAP levels in four cortical regions and the hippocampus using an ELISA technique developed by NIOSH scientists in all HAAS archive brains with frozen tissue available. Analyses with these data have demonstrated significantly high levels of GFAP in the temporal, parietal, and occipital cortices (but not the frontal cortex) in Alzheimer's disease brains compared to age matched controls.(14) Work is currently underway to perform the same assay on frozen samples of caudate and putamen and to examine associations of GFAP levels in these regions with Parkinson's disease and parkinsonism. The availability of these GFAP levels presents an excellent opportunity to examine the association of brain organochlorine levels with this sensitive measure of brain injury.

Although possible effects of organochlorine compounds on cognition have not been generally recognized, it is reasonable to hypothesize that organochlorine toxicity could cause cognitive and

behavioral dysfunction. One of the primary goals of the HAAS is to identify and sub-classify all cases of dementia in the cohort. All participants receive cognitive screening during HAAS examinations using the cognitive abilities screening instrument (CASI). Neuropathological evaluations include a comprehensive standardized gross and microscopic examination of the brain that includes neurofibrillary tangle and neuritic plaque counts in multiple cortical and hippocampal regions. The availability of these data will allow us to examine the association of brain organochlorine levels with clinical endpoints including CASI score as a general measure of cognitive function, as well as dementia clinical diagnoses. Finally, neuropathological indicators of dementia including neurofibrillary tangles and neuritic plaques will be examined for a relationship with brain organochlorines.

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APPENDIX 1

BRAIN TISSUE ANALYSIS IN THE HONOLULU-ASIA AGING STUDY (HAAS) - PESTICIDES AND OTHER PERSISTENT CHEMICALS. D.B. Miller1, G.W. Ross2,4, J. P. O'Callaghan1, M. L. Kashon1, C. M. Burchfiel1, D. S. Sharp1, E. D. Pellizzari,3, H. Petrovitch4, W. Sanderson5, L. R. White4, 1Health Effects Laboratory Division, CDC-NIOSH, Morgantown, WV, 2Department of Veteran's Affairs, Honolulu, HI, 3Research Triangle Institute, RTP, NC, 4University of Iowa, Iowa City, IA,5Pacific Health Research Institute, Honolulu, HI

The environmental etiology of neurodegenerative disorders is of great research interest and pesticides have received special scrutiny because of their pervasive use in modern agriculture. The HAAS is a cohort of 8,006 Japenese-American men for whom extensive lifestyle, work, and health records have been collected since the late 1960s. Collection at autopsy of unfixed brain material from a subset of this group allows the measurement of brain levels of persistent chemicals in this cohort. Records from the Department of Agriculture were used to select compounds with specific agricultural/industrial uses in Hawaii. Organochlorines have been used extensively in Hawaii since the 1940s (e.g., DDT) and by the 1960s 4 of the most commonly used on plantations were chlordane, DDT, heptachlor, and lindane (BHC gamma). A capillary GC method with electron capture capable of detection in the parts per billion (PPB) range will be used to assay 80 mg of brain cortex from ~200 men for these 4 organochlorines as well as DDT and its metabolite DDE, oxychlordane, trans-nonachlor, dieldrin, BHC (alpha & beta), HCB, endrin, methoxychlor and mirex among others. A pilot evaluation of 15 men with an extensive history of plantation work found measurable levels of compounds with specific agricultural/industrial use (e.g., dieldrin, methoxychlor, trans-nonachlor) as well as compounds with more general usage (e.g., DDE, oxychlordane, g-chlordane). Levels ranged from 1.8 - 13.24, 2.13 - 26.05, 1.62 - 7.9, 2.66 - 93.66, 2.11 - 10.3, 4.17 - 12.38, PPB for dieldrin, methoxychlor, trans-nonachlor, DDE, oxychlordane and g-chlordane, respectively. These compounds deposit in lipids and the percentage of lipids in the brain tissue analyzed ranged from 1.8 - 7.1% of the tissue weight (24 - 104 mg). The lowest quantifiable limit or the lowest level of compound that can be detected with confidence by the method of analysis is 1.56 PPB for all compounds except methoxychlor for which the limit is 7.81 PPB. Analysis of brain tissue from the HAAS allows for determination of the relations between persistent chemicals in brain and degenerative disorders such as Parkinson's and Alzheimer's diseases.

APPENDIX 2

Method for the Determination of Organochlorine Pesticides in Human Brain Tissue

B. Childs[1], K. Briggs[1], E. Pellizzari1, J. Beach, W. Ross[2,3], H Petrovitch[2,3] and D. Miller[3].
[1]RTI, International, POB 12194, Research Triangle Park, NC 27709; [2]Honolulu Dept of Veterans Affairs; [3]Pacific Health Research Institute, 846 S. Hotel St., Suite 306, Honolulu, HI 96813; [3]CDC-NIOSH, 1600 Clifton Rd., Atlanta, GA 30333

Because organochlorine pesticides are persistent in the environment and in the body, and the nature of their toxicity, there continues to be interest in their occurrence. Epidemiological studies have linked environmental risk factors such as rural living, pesticide use, and Parkinson's disease. In addition, there have been reports of these persistent pesticides in postmortem human brain. For these reasons, a study is underway on the measurement of organochlorine pesticides in autopsy brain samples from Japanese-American men born between 1900 and 1919 and living on Oahu, HI when the study began in 1965 Exposures occurring during the application of pesticides during rural agricultural practices are hypothesized to be elevated in these subjects.

A gas chromatographic-electron capture detection (GC-ECD) method was developed and applied to the analysis of milligram quantities of human brain tissue for aldrin, α-BHC, β-BHC, γ-BHC, α-chlordane, γ-chlordane, DDE, DDD, DDT, dieldrin, endrin, endosulfan-1, endosulfan-2, HCB, heptachlor, hepox-b, isobenzan, oxchlordane, methoxchlor, mirex, and *t*-nonachlor. Briefly, this consists of adding a surrogate compound to 25-30 mg of tissue, grinding the tissue with anhydrous sulfate, extracting with hexane, and purifying the pesticides on a column of partially deactivated Florisil. An external standard is added to the three concentrated fractions collected from the Florisil column containing the pesticides of interest and the fractions are analyzed by capillary GC-ECD. The figures of merit for the method included, determining (1) the average percent recoveries of the pesticides in method controls for eleven sets of samples, (2) the precision of analysis for duplicate samples, and (3) background levels in blanks. Details and performance of the method will be presented. The method is being applied to over 400 brain samples from deceased Japanese-American subjects.

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